PCT/JP2004/004596
30. 4. 2004

PA 1150056

THE UNITED STATES OF AMERICA

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April 06, 2004

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APPLICATION NUMBER: *60/458,370*
FILING DATE: *March 31, 2003*

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN
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COMMISSIONER OF PATENTS AND TRADEMARKS

P. SWAIN
Certifying Officer

60458370 03/31/03

PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

Docket Number 236048US0PROV

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☒ Additional inventors are named on separately numbered sheets attached hereto.

TITLE OF THE INVENTION (280 CHARACTERS MAX)

METHOD FOR TREATING DISEASE CAUSED/ACCOMPANIED BY INCREASED VASCULAR PERMEABILITY

CORRESPONDENCE ADDRESS

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ENCLOSED APPLICATION PARTS

■ Specification *Number of Pages:* 19 ☐ CD(s), Number

☐ Drawing(s) *Number of Sheets:* ■ Other (specify): Application Data Sheet; White Advance Serial Number Card

METHOD OF PAYMENT

☐ Applicant claims small entity status. See 37 CFR 1.27.

■ A check or money order is enclosed to cover the Provisional Filing Fees

■ The Commissioner is hereby authorized to charge filing fees and credit any overpayment to Deposit Account Number 15-0030

PROVISIONAL FILING FEE AMOUNT $160.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

■ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

3/31/03
DATE

Respectfully Submitted,

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PROVISIONAL APPLICATION FILING ONLY

PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

			Docket Number 236048US0PROV
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APPLICATION DATA SHEET

APPLICATION INFORMATION

Application Type::	PROVISIONAL
Subject Matter::	UTILITY
CD-ROM or CD-R?::	NONE
Title::	METHOD FOR TREATING DISEASE CAUSED/ACCOMPANIED BY INCREASED VASCULAR PERMEABILITY
Attorney Docket Number::	236048us

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CORRESPONDENCE INFORMATION

Correspondence Customer Number::	22850

REPRESENTATIVE INFORMATION

Representative Customer Number:: 22850

Initial 03/31/03

SPECIFICATION

METHOD FOR TREATING DISEASE CAUSED/ACCOMPANIED BY INCREASED VASCULAR PERMEABILITY

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a method for treating a disease caused/accompanied by increased vascular permeability.

BACKGROUND OF THE INVENTION

Vascular adhesion protein-1 (hereinafter to be abbreviated as VAP-1) is amine oxidase (semicarbazide sensitive amine oxidase, SSAO) which is abundant in human plasma, and shows remarkably increased expression in vascular endothelium and vascular smooth muscle of the inflammatory region. While the physiological role of VAP-1 has not been clarified until recently, VAP-1 gene was cloned in 1998, and VAP-1 has been reported to be a membrane protein that regulates rolling and migration of lymphocyte and NK cell as an adhesion molecule under regulation of expression by inflammatory cytokine. Although the amine to be a substrate is unknown, it is considered to be methylamine generated in any part of living organisms. It is also known that hydrogen peroxide and aldehydes produced by the amine oxidase activity in a molecule are important factors of adhesion activity.

However, the correlation between the VAP-1 enzyme activity in plasma and vascular permeability has not been heretofore known.

SUMMARY OF THE INVENTION

The present inventors have found that VAP-1 enzyme activity in plasma and vascular permeability are correlated, and therefore, a VAP-1 inhibitor is useful for the prophylaxis or treatment of a disease caused/accompanied by increased vascular permeability and completed the present invention. Thus, the present invention provides the

following:

(1) A method for treating a disease caused/accompanied by increased vascular permeability, which method comprises administering to a subject in need thereof a vascular adhesion protein-1 (VAP-1) inhibitor in an amount sufficient to treat said subject for said disease.

(2) The method of (1), wherein said disease is a disease in mucous membrane.

(3) The method of (2), wherein said mucous membrane is a mucous membrane of ocular, cutis, otorhinology or respiratory tract.

(4) The method of (1), wherein said disease is aged macular degeneration, aged disciform macular degeneration, cystoid macular edema, palpebral edema, uveitis, conjunctivitis, cyclitis, scleritis, episcleritis, optic neuritis, retrobulbar optic neuritis, keratitis, blepharitis, exudative retinal detachment, corneal ulcer, conjunctival ulcer, chronic nummular keratitis, Thygeson keratitis, progressive Mooren's ulcer, an ocular inflammatory disease caused by an ophthalmic operation, an ocular inflammatory disease caused by a physical injury to the eye, a symptom caused by an ocular inflammatory disease including itching, flare, edema and ulcer, erythema, erythema exsudativum multiforme, erythema nodosum, erythema annulare, scleredema, dermatitis, angioneurotic edema, laryngeal edema, glottic edema, subglottic laryngitis, bronchitis, rhinitis, pharyngitis, sinusitis, laryngitis or otitis media.

(5) The method of (1), wherein the VAP-1 inhibitor is N-{4-[2-(4-{[amino(imino)methyl]amino}phenyl)ethyl]-1,3-thiazol-2-yl}acetamide or a derivative thereof, or a pharmaceutically acceptable salt thereof.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is predicated on the discovery that

that VAP-1 enzyme activity in plasma and vascular permeability are correlated, and therefore, an inhibitor of vascular adhesion protein-1 (VAP-1; also referred to as semicarbaside sensitive amine oxidase (SSAO) or copper-containing amine oxidase) is effective in treating or ameliorating a disease caused/accompanied by increased vascular permeability. Accordingly, the present invention provides a method for treating a disease caused/accompanied by increased vascular permeability. The "treating a disease caused/accompanied by increased vascular permeability" and "treatment of a disease caused/accompanied by increased vascular permeability" are intended to include the administration of a compound having a VAP-1 inhibitory activity to a subject for purposes, which can include prophylaxis, amelioration, prevention and cure of a disease caused/accompanied by increased vascular permeability. As used herein, by the "subject" is meant a target of the administration of VAP-1 inhibitor in the present invention, which is specifically any animal such as human, mouse, rat, swine, dog, cat, horse, bovine and the like, especially human. The disease caused/accompanied by increased vascular permeability, which is to be treated by the method of the present invention, includes, for example, diseases in mucous membrane such as ocular, cutis, otorhinology, respiratory tract and the like. Examples thereof include diseases in ocular-mucous membrane, such as aged macular degeneration, aged disciform macular degeneration, cystoid macular edema, palpebral edema, uveitis, conjunctivitis, cyclitis, scleritis, episcleritis, optic neuritis, retrobulbar optic neuritis, keratitis, blepharitis, exudative retinal detachment, corneal ulcer, conjunctival ulcer, chronic nummular keratitis, Thygeson keratitis, progressive Mooren's ulcer, ocular inflammatory diseases caused by an ophthalmic operation, ocular inflammatory diseases caused by a physical injury to

the eye and symptoms caused by ocular inflammatory diseases including itching, flare, edema and ulcer; mucocutaneous diseases, such as erythema, erythema exsudativum multiforme, erythema nodosum, erythema annulare, scleredema, dermatitis and angioneurotic edema; and diseases in mucous membrane (e.g., otorhinology, respiratory tract etc.), such as laryngeal edema, glottic edema, subglottic laryngitis, bronchitis, rhinitis, pharyngitis, sinusitis, laryngitis and otitis media.

The method comprises the administration of a VAP-1 inhibitor in an amount sufficient to treat a disease caused/accompanied by increased vascular permeability. Any VAP-1 inhibitor can be used in the method of the present invention as long as it is safe and efficacious. Herein, the "VAP-1 inhibitor" will be used to refer to such compounds and is intended to encompass all compounds that inhibit enzyme activity of VAP-1 at any and all points in the action mechanism thereof.

For example, a novel compound N-{4-[2-(4-{[amino(imino)methyl]amino}phenyl)ethyl]-1,3-thiazol-2-yl}acetamide and derivatives thereof, or compounds reported to have inhibited VAP-1 enzyme (SSAO) may include fluoroallylamine derivatives, semicarbazide derivatives, hydrazide derivatives, hydrazino derivatives, 1,3,4-oxadiazine derivatives, 2,6-diethoxybenzylamine, 2,6-di(n-propoxy)benzylamine, 2,6-diisopropoxybenzylamine, 2,6-di(n-butoxy)benzylamine, 2,6-bis(methoxymethoxy)benzylamine, 2,6-bis(methoxymethyl)benzylamine, 2,6-diethylbenzylamine, 2,6-di-n-propylbenzylamine, 2,6-bis(2-hydroxyethoxy)benzylamine, and the like.

The above compounds can be illustrated as follows.

1) fluoroallylamine derivatives, semicarbazides derivatives and hydrazides derivatives described in WO93/23023,

2) hydrazino derivatives described in WO02/02090,

3) 1,3,4-oxadiazine derivatives described in WO02/02541,

4) 4-alkyl-5-alkoxycarbonyl-4,5,6,7-tetrahydroimidazo[4,5-c]pyridine derivatives described in WO02/38153,

5) 2,6-diethoxybenzylamine, 2,6-di(n-propoxy)benzylamine, 2,6-diisopropoxybenzylamine, 2,6-di(n-butoxy)benzylamine, 2,6-bis(methoxymethoxy)benzylamine, 2,6-bis(methoxymethyl)benzylamine, 2,6-diethylbenzylamine, 2,6-di-n-propylbenzylamine and 2,6-bis(2-hydroxyethoxy)benzylamine described in US4,888,283.

The compounds exemplified in the present invention as a VAP-1 inhibitor and in WO93/23023 as an SSAO inhibitor, such as those described in Lyles et al. (1987 Biochem. Pharmacol. 36:2847) and in USP 4650907, USP 4916151, USP 4943593, USP 4965288, USP 5021456, USP 5059714, USP 4699928, European patent application 295604, European patent application 224924 and European patent application 168013, are also encompassed in the VAP-1 inhibitor of the present invention. Of the above-mentioned compounds, preferred are N-{4-[2-(4-{[amino(imino)methyl]amino}phenyl)ethyl]-1,3-thiazol-2-yl}acetamide and derivatives thereof.

The term "derivative" is intended to include all compounds derived from the original compound.

In the present invention, the VAP-1 inhibitor can be administered to a subject as a prodrug. The term "prodrug" is intended to include all compounds that convert to the VAP-1 inhibitor in the body of administration subject. The prodrug can be any pharmaceutically acceptable prodrug of VAP-1 inhibitor. Moreover, the VAP-1 inhibitor of the present invention can be administered to a subject of the administration as a pharmaceutically acceptable salt.

The pharmaceutically acceptable salt of VAP-1 inhibitor in the present invention is nontoxic and pharmaceutically acceptable conventional salts, which are exemplified by salts

with inorganic or organic base such as alkali metal salt (e.g., sodium salt, potassium salt and the like), alkaline earth metal salt (e.g., calcium salt, magnesium salt and the like), ammonium salt, and amine salt (e.g., triethylamine salt, N-benzyl-N-methylamine salt and the like).

The VAP-1 inhibitor can be also formulated as a pharmaceutically acceptable acid addition salt. Examples of pharmaceutically acceptable acid addition salts for use in the pharmaceutical composition include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric and sulfuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, and arylsulphonic, for example p-toluenesulphonic, acids.

The above-mentioned VAP-1 inhibitor may be commercially available or can be produced based on a known reference. The novel compound, N-{4-[2-(4-{[amino(imino)methyl]amino}phenyl)ethyl]-1,3-thiazol-2-yl}acetamide, can be synthesized according to the production examples given below. Those compounds or derivatives thereof that are not commercially available can be readily prepared using organic synthesis methods known in the art.

The compound having VAP-1 inhibitory activity or a pharmaceutically acceptable salt thereof can be administered in accordance with the present inventive method by any suitable route. Suitable routes of administration include systemic, such as orally or by injection, topical, periocular (e.g., subTenon's), subconjunctival, intraocular, subretinal, suprachoroidal, and retrobulbar administrations. The manner in which the VAP-1 inhibitor is administered is dependent, in part, upon whether the treatment of a disease caused/accompanied by increased vascular permeability is prophylactic or therapeutic.

The VAP-1 inhibitor is preferably administered as soon as possible after it has been determined that a subject such as a mammal, specifically a human, is at risk for a disease caused/accompanied by increased vascular permeability (prophylactic treatments) or has begun to develop a disease caused/accompanied by increased vascular permeability (therapeutic treatments). Treatment will depend, in part, upon the particular VAP-1 inhibitor used, the amount of the VAP-1 inhibitor administered, the route of administration, and the cause and extent, if any, of a disease caused/accompanied by increased vascular permeability realized.

One skilled in the art will appreciate that suitable methods of administering a VAP-1 inhibitor, which is useful in the present inventive method, are available. Although more than one route can be used to administer a particular VAP-1 inhibitor, a particular route can provide a more immediate and more effective reaction than another route. Accordingly, the described routes of administration are merely exemplary and are in no way limiting.

The dose of the VAP-1 inhibitor administered to the administration subject such as animal including human, particularly a human, in accordance with the present invention should be sufficient to effect the desired response in the subject over a reasonable time frame. One skilled in the art will recognize that dosage will depend upon a variety of factors, including the strength of the particular VAP-1 inhibitor employed, the age, species, condition or disease state, and body weight of the subject, as well as the degree of a disease caused/accompanied by increased vascular permeability. The size of the dose also will be determined by the route, timing and frequency of administration as well as the existence, nature, and extent

of any adverse side effects that might accompany the administration of a particular VAP-1 inhibitor and the desired physiological effect. It will be appreciated by one of ordinary skill in the art that various conditions or disease states, may require prolonged treatment involving multiple administrations.

Suitable doses and dosage regimens can be determined by conventional range-finding techniques known to those of ordinary skill in the art. Generally, treatment is initiated with smaller dosages, which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached.

Generally, the VAP-1 inhibitor can be administered in the dose of from 0.001 μg/kg/day to about 300 mg/kg/day, preferably from about 0.01 μg/kg/day to about 10 mg/kg/day, which is given in a single dose or 2 to 4 doses a day or in a sustained manner.

Compositions for use in the present inventive method preferably comprise a pharmaceutically acceptable carrier and an amount of a VAP-1 inhibitor sufficient to treat a disease caused/accompanied by increased vascular permeability prophylactically or therapeutically. The carrier can be any of those conventionally used and is limited only by chemico-physical considerations, such as solubility and lack of reactivity with the compound, and by the route of administration.

The amount of the VAP-1 inhibitor in the composition may vary depending on the formulation of the composition, and may generally be 0.00001 - 10.0 wt%, preferably 0.0001 - 5 wt%, more preferably 0.001 - 1 wt%.

The VAP-1 inhibitor can be administered in various manners to achieve the desired VAP-1 inhibitory effect. The

VAP-1 inhibitors can be administered alone or in combination with pharmaceutically acceptable carriers or diluents, the properties and nature of which are determined by the solubility and chemical properties of the inhibitor selected, the chosen route administration, and standard pharmaceutical practice. VAP-1 inhibitor may be administered orally in solid dosage forms, e.g. capsules, tablets, powders, or in liquid forms, e.g. solutions or suspensions. The inhibitors may also be injected parenterally in the form of sterile solutions or suspensions. Solid oral forms may contain conventional excipients, for instance: lactose, sucrose, magnesium stearate, resins, and like materials. Liquid oral forms may contain various flavoring, coloring, preserving, stabilizing, solubilizing, or suspending agents. Parenteral preparations are sterile aqueous or non-aqueous solutions or suspensions which may contain certain various preserving, stabilizing, buffering, solubilizing, or suspending agents. If desired, additives, such as saline or glucose, may be added to make the solutions isotonic.

VAP-1 inhibitor may also be topically administered to such locations as eyes, skin, respiratory duct, nasal cavity, ear, labial, pubis and pudenda.

In the case of topically administering a formulation, the formulation manufactured by conventional methods may be administered, which includes all the formulations for topical ocular administration used in the field of ophthalmology (e.g., eye drops and eye ointment) and all the formulations for external use in the fields of dermatology and otolaryngology (e.g., ointment, cream, lotion and spray).

The eye drops are prepared by dissolving the active ingredient in a sterile aqueous solution such as physiological saline, buffering solution, etc., or by combining powder compositions to be dissolved before use. If

desired, additives ordinarily used in the eye drops can be added. Such additives include isotonizing agents (e.g., sodium chloride, etc.), buffer agent (e.g., boric acid, sodium monohydrogen phosphate, sodium dihydrogen phosphate, etc.), preservatives (e.g., benzalkonium chloride, benzethonium chloride, chlorobutanol, etc.), thickeners (e.g., saccharide such as lactose, mannitol, maltose, etc.; e.g., hyaluronic acid or its salt such as sodium hyaluronate, potassium hyaluronate, etc.; e.g., mucopolysaccharide such as chondroitin sulfate, etc.; e.g., sodium polyacrylate, carboxyvinyl polymer, crosslinked polyacrylate, etc.). The disclosure of the above publication is incorporated herein by reference.

The ointment (including eye ointment) is prepared by mixing the active ingredient with the base. The formulation can be prepared according to the ordinary method. For example, mixing the active ingredient into the base ordinarily used for the ointment and formulating it according to ordinary methods can sterilely prepare the ointment. Examples of the base for the ointment include petrolatum, selen 50, Plastibase, macrogol, etc., but not limited thereto. Further, in order to increase the hydrophilicity, a surface-active agent can be added. Regarding the ointment, the above-mentioned additives such as the preservatives, etc. can be combined, if necessary.

The present agent can be formulated as a sterile unit dose type containing no preservatives.

The present inventive method also can involve the co-administration of other pharmaceutically active compounds. By "co-administration" is meant administration before, concurrently with, e.g., in combination with the VAP-1 inhibitor in the same formulation or in separate formulations, or after administration of a VAP-1 inhibitor

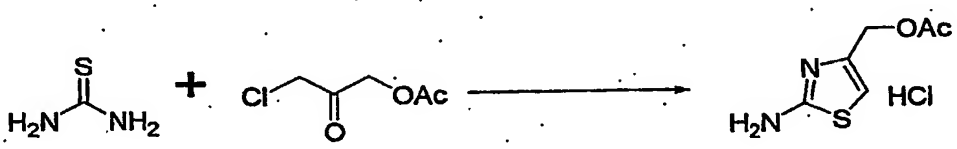
as described above. For example, corticosteroids, prednisone, methylprednisolone, dexamethasone, or triamcinalone acetinide, or noncorticosteroid anti-inflammatory compounds, such as ibuprofen or flubiproben, can be co-administered. Similarly, vitamins and minerals, e.g., zinc, anti-oxidants, e.g., carotenoids (such as a xanthophyll carotenoid like zeaxanthin or lutein), and micronutrients can be co-administered.

The present invention is explained in more detail in the following by way of Production Example and Example, which are not to be construed as limitative.

The test compound used in the Example was N-{4-[2-(4-{[amino(imino)methyl]amino}phenyl)ethyl]-1,3-thiazol-2-yl}acetamide (hereinafter Compound A) synthesized in Production Example.

Production Example

Step 1:

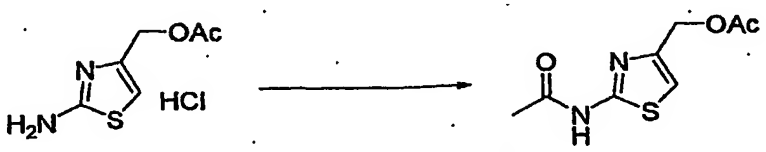


A mixture of 3-chloro-2-oxopropyl acetate (5 g) and thiourea (2.5 g) in ethanol (25 ml) was refluxed for 4 hours. The reaction mixture was cooled to ambient temperature and the resulting crystalline precipitate was collected by filtration and washed with ethanol (20 ml) to give (2-amino-1,3-thiazol-4-yl)methyl acetate hydrochloride (3.5 g) as white crystals.

NMR(DMSO-d_6, δ) 2.07(3H, s), 4.92(2H, s), 6.87(1H, s)

MS: 173$(M+H)^+$

Step 2

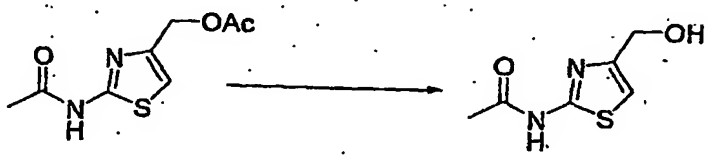


To a mixture of (2-amino-1,3-thiazol-4-yl)methyl acetate hydrochloride (56 g) and pyridine (45 g) in dichloromethane (560 ml) was added acetyl chloride (23 g) over a period of 30 minutes at 5°C and the reaction mixture was stirred for 10 minutes at the same temperature. The reaction mixture was poured into water (500 ml) and extracted with chloroform (1 L). The organic layer was dried over sodium sulfate and concentrated *in vacuo*. The residual solid was collected by filtration with isopropyl ether to give (2-(acetylamino)-1,3-thiazol-4-yl)methyl acetate (47 g) as white crystals.

NMR($CDCl_3$, δ) 2.12(3H, s), 2.29(3H, s), 5.08(2H, s), 6.93(1H, s)

MS: 215$(M+H)^+$

Step 3

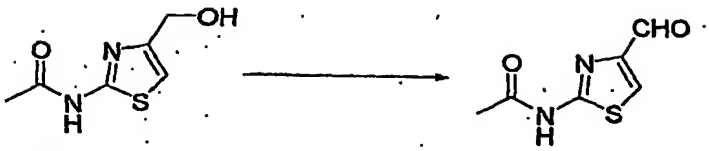


A mixture of (2-(acetylamino)-1,3-thiazol-4-yl)methyl acetate (46 g) and potassium carbonate (30 g) in methanol (640 ml) was stirred for 3 hours at ambient temperature. The reaction mixture was concentrated *in vacuo*. The residue was diluted with chloroform and the insoluble material was filtered off. The resulting solution was purified by flash column chromatography on silica-gel with methanol/chloroform (1/99). The resulted solid was collected by filtration with isopropyl ether to give N-(4-(hydroxymethyl)-1,3-thiazol-2-yl)acetamide (35 g) as white crystals.

NMR(DMSO-d_6, δ) 2.12(3H, s), 4.44(2H, d, J=5.0Hz), 5.20(1H, t, J=5.0Hz), 6.88(1H, s), 12.02(1H, brs)

MS: 173$(M+H)^+$

Step 4

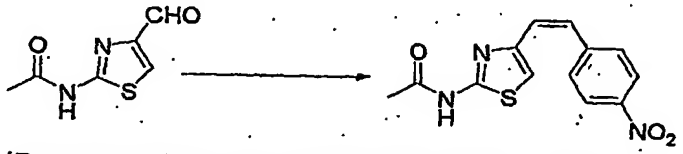


N-(4-(Hydroxymethyl)-1,3-thiazol-2-yl)acetamide (2.8 g) was dissolved in methanol (10 ml) and chloroform (200 ml). Then manganese (IV) oxide (28.3 g) was added to the solution under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 7 hours, and filtered through a celite pad. The filtrate was concentrated *in vacuo*. The resulting solid was washed with ethyl ether to give N-(4-formyl-1,3-thiazol-2-yl)acetamide (2.01 g) as an off-white solid.

mp. 195.5-199°C.

NMR(DMSO-d_6, δ) 2.17(3H, s), 8.28(1H, s), 9.79(1H, s), 12.47(1H, brs).

Step 5



1-(Bromomethyl)-4-nitrobenzene (1.9 g), triphenylphosphine (2.31 g) and N,N-dimethylformamide (20 ml) were combined under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 2.5 hours. Then potassium tert-butoxide (1.19 g) and N-(4-formyl-1,3-thiazol-2-yl)acetamide (1.5 g) were added to the mixture, and stirred at room temperature for 14 hours. The reaction mixture was poured into ice-water, and extracted with ethyl acetate. The organic layer was washed with 1N-hydrochloric acid, water and saturated sodium chloride solution, dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was purified by flash column chromatography over silica gel with an n-hexane / ethyl acetate (1:1) → (1:2) as eluant, and triturated with ethyl ether to give N-{4-[(Z)-2-(4-

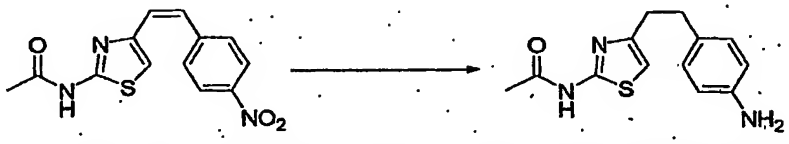
nitrophenyl)ethenyl]-1,3-thiazol-2-yl}acetamide (1.59 g) as a yellow solid.

mp. 155-157°C

NMR(DMSO-d_6, δ) 2.13(3H, s), 6.64(1H, d, J=12.5Hz), 6.71(1H, d, J=12.5Hz), 7.18(1H, s), 7.79(2H, d, J=9.0Hz), 8.17(2H, d, J=9.0Hz), 12.02(1H, brs).

MS: 290$(M+H)^+$

Step 6



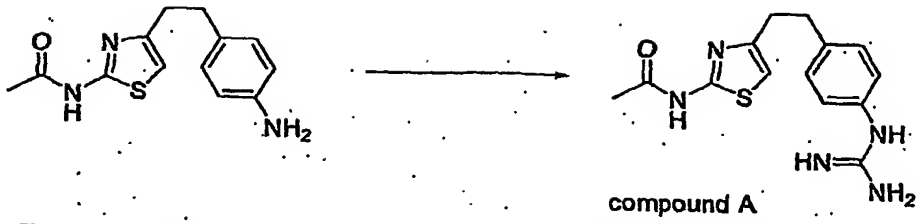
A mixture of N-{4-[(Z)-2-(4-nitrophenyl)ethenyl]-1,3-thiazol-2-yl}acetamide (2 g) and 10% palladium carbon (400 mg) in methanol (25 ml), tetrahydrofuran (25 ml) and acetic acid (18 ml) was stirred under 4 atm hydrogen at ambient temperature for 5 hours. The reaction mixture was filtered through a celite pad, and the filtrate was concentrated *in vacuo*. The residue was dissolved in ethyl acetate. The organic solution was washed with saturated sodium hydrogen carbonate solution and saturated sodium chloride solution, dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was purified by flash column chromatography over silica gel with an n-hexane / ethyl acetate (1:2) → ethyl acetate as eluant, and triturated with ethyl alcohol / ethyl ether to give N-(4-(2-(4-aminophenyl)ethyl)-1,3-thiazol-2-yl)acetamide (539.6 mg) as an off-white solid.

mp. 102.5-104°C

NMR(DMSO-d_6, δ) 2.11(3H, s), 2.75(4H, brs), 4.82(2H, s), 6.46(2H, d, J=8.5Hz), 6.69(1H, s), 6.83(2H, d, J=8.5Hz), 12.07(1H, brs).

MS: 262$(M+H)^+$

Step 7



compound A

To a suspension of N-(4-(2-(4-aminophenyl)ethyl)-1,3-thiazol-2-yl)acetamide (26 g) in ethanol (500 ml) was added 4N hydrogen chloride in ethyl acetate (25 ml) and cyanamide (6.3 g). The mixture was refluxed for 26 hours. The reaction mixture was cooled to ambient temperature and poured into a mixture of ethyl acetate (500 ml) and saturated sodium hydrogencarbonate solution (500 ml). The resulted precipitate was collected by filtration and washed with water (300 ml) and ethanol (300 ml) to give N-{4-[2-(4-{[amino(imino)methyl]amino}phenyl)ethyl]-1,3-thiazol-2-yl}acetamide (18 g) as white crystals.

NMR(DMSO-d_6, δ) 2.10(3H, s), 2.85(4H, s), 6.79(1H, s), 6.83(2H, d, J=7Hz), 7.10(2H, d, J=7Hz)

MS: 304 $(M+H)^+$

Example

Inhibitory Effect of Compound A on VAP-1 enzyme (SSAO) activity in human and rat plasma.

VAP-1 enzyme (SSAO) activity in both human and rat plasma was determined by a radiochemical-enzyme assay using ^{14}C-benzylamine as artificial substrate. The enzyme suspension prepared from blood plasma was pre-incubated with Compound A in 96-well microplate at room temperature for 30 min. The enzyme suspension was then incubated with ^{14}C-benzylamine ($2x10^{-5}$ mol/l final concentration) in a final volume of 50 µl at 37°C for 1 hour. The enzyme reaction was terminated by adding 2 mol/l (50 µl) citric acid. The oxidized products were directly extracted into a 200 µl toluene scintillator, and its radioactivity was measured by a scintillation spectrometer.

Monoamine oxidase (MAO) and diamine oxidase (DAO, histaminase) activities were also determined by similar method using ^{14}C-phenylethylamine and ^{14}C-putrescine as substrate, respectively. Cloned DAO from cDNA libraries was used in human DAO assay. Inhibition activity was expressed as IC_{50} (μmol/l) value.

Compound A completely inhibited the enzyme activity of human and rat plasma SSAO, but not the enzyme activities of other amine oxidases, such as human platelet MAO and cloned DAO, shown in Table 1.

Table 1. Inhibitory effect (IC_{50} values, μM) of Compound A on various amine oxidase activities

Human plasma SSAO	Rat plasma SSAO	Human platelet MAO	Cloned Human DAO
0.15	0.012	>100	>100

WHAT IS CLAIMED IS

1. A method for treating a disease caused/accompanied by increased vascular permeability, which method comprises administering to a subject in need thereof a vascular adhesion protein-1 (VAP-1) inhibitor in an amount sufficient to treat said subject for said disease.

2. The method of claim 1, wherein said disease is a disease in mucous membrane.

3. The method of claim 2, wherein said mucous membrane is a mucous membrane of ocular, cutis, otorhinology or respiratory tract.

4. The method of claim 1, wherein said disease is aged macular degeneration, aged disciform macular degeneration, cystoid macular edema, palpebral edema, uveitis, conjunctivitis, cyclitis, scleritis, episcleritis, optic neuritis, retrobulbar optic neuritis, keratitis, blepharitis, exudative retinal detachment, corneal ulcer, conjunctival ulcer, chronic nummular keratitis, Thygeson keratitis, progressive Mooren's ulcer, an ocular inflammatory disease caused by an ophthalmic operation, an ocular inflammatory disease caused by a physical injury to the eye, a symptom caused by an ocular inflammatory disease including itching, flare, edema and ulcer, erythema, erythema exsudativum multiforme, erythema nodosum, erythema annulare, scleredema, dermatitis, angioneurotic edema, laryngeal edema, glottic edema, subglottic laryngitis, bronchitis, rhinitis, pharyngitis, sinusitis, laryngitis or otitis media.

5. The method of claim 1, wherein the VAP-1 inhibitor is N-(4-

[2-(4-{[amino(imino)methyl]amino}phenyl)ethyl]-1,3-thiazol-2-yl}acetamide or a derivative thereof, or a pharmaceutically acceptable salt thereof.

ABSTRACT OF THE DISCLOSURE

The present invention provides a method for treating a disease caused/accompanied by increased vascular permeability, which method comprises administering to a patient in need thereof a vascular adhesion protein-1 (VAP-1) inhibitor in an amount sufficient to treat said patient for said disease.

DOCKET NO.: 278283US0X PCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: Ryuji UENO, et al.
SERIAL NO.: NEW U.S. PCT APPLICATION
FILED: HEREWITH
INTERNATIONAL APPLICATION NO.: PCT/JP04/04596
INTERNATIONAL FILING DATE: March 31, 2004
FOR: METHOD FOR TREATING VASCULAR HYPERPERMEABLE DISEASE

**REQUEST FOR PRIORITY UNDER 35 U.S.C. 119(e)
AND THE INTERNATIONAL CONVENTION**

Commissioner for Patents
Alexandria, Virginia 22313

Sir:

In the matter of the above-identified application for patent, notice is hereby given that the applicant claims as priority:

COUNTRY	APPLICATION NO	DAY/MONTH/YEAR
USA	60/458,370	31 March 2003

Certified copies of the corresponding Convention application(s) were submitted to the International Bureau in PCT Application No. PCT/JP04/04596. Receipt of the certified copy(s) by the International Bureau in a timely manner under PCT Rule 17.1(a) has been acknowledged as evidenced by the attached PCT/IB/304.

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